UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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SEP 11 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA registration number: 264-346; 2-(2,4 dichlorophenoxy) propionic acid [2,4-DP, dichlorprop, dichloroprop]; chronic rat study.

Caswell number: 320.

Accession number: 255729-255732.

TO: Richard Mountfort
Product Manager (23)
Registration Division (TS-767).

THRU: R. Bruce Jaeger
Head, Review Section I
Toxicology Branch (TS-769)
Hazard Evaluation Division

FROM: Van M. Seabaugh
Toxicology Branch (TS-769)
Hazard Evaluation Division

1. Chemical:

2-(2-4 dichlorophenoxy) propionic acid [2,4-DP, dichlorprop, dichloroprop]; Caswell number: 320.

2. Test Material Number:

Name:	2,4-DP acid.
Purity:	95% (lot number: 9-22-80) 5/19/81 - 1/31/82.
	95% (lot number: 10-31-80) 2/1/82 - 6/25/83.
Manufacturer:	Not stated.
Description:	White crystalline solid.
Melting point:	116-117.5° C.
Solubility:	Slightly soluble in water (0.71 g/l water).
	Soluble in acetone and benzene.
Stability:	Stable at room temperature.
Storage Condition:	Dark room; 4°C.

3. Study/Action Type:

Chronic toxicity feeding study for two years in SPF Fisher rats (F-344) of both sexes.

4. Study Identification:

Chronic toxicity feeding study in rats; full study conducted by The Institute Of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan for Union Carbide Agricultural Products Co., Inc., P.O. Box 12014, T.W. Alexander Dr., Research Triangle Park, N.C., 27709.

5. Reviewed By:

Van M. Seabaugh
Toxicology Branch (TS-769)
Hazard Evaluation Division

6. Approved By:

R. Bruce Jaeger
Head, Review Section I
Toxicology Branch (TS-769).
Hazard Evaluation Division

7. Study Protocol: See attachment (appendix 1).

8. Conclusions:

A. Previously submitted feeding studies by Union Carbide are as follows: 1) 24 month Sprague Dawley Rat Oncology Study (0, 500, 1,000, 4,000/ 3,000 ppm). EPA conclusion: carcinogenic in male rats (EPA accession numbers: 244476-244481; see details on page 9 of the present report). 2) 18 month oncology study in Swiss-Webster (CD-1) mice (0, 25, 100, and 300 mg/kg). EPA conclusion: not carcinogenic (EPA accession numbers 242035-242038).

B. Present study. 2,4-DP acid was fed in the diets of SPF Fisher rats (F-344) of both sexes for 24 months at treatment groups of 0, 100, 300, 1,000 and 3,000 ppm. There were eighty rats of each sex per treatment group. Interim sacrifice and examination for urinalysis, hematology, blood chemistry, and pathology (8 males and 8 females per group) were after 26, 52, and 78 weeks of treatment. Hematological and other biochemical examinations were conducted at the end of 104 weeks of treatment. All animals killed in extremis or found dead had pathological examinations. It is concluded that 2,4-DP

acid is not a carcinogen in SPF Fisher Rats (F-344). Tumors (neoplastic lesions) were not significantly increased compared to the controls.

Incidence Of Tumors Excluding Animals Subjected To Interim Kill

Tumors	Dose Groups (ppm)									
	0		100		300		1,000		3,000	
Sex:	M	F	M	F	M	F	M	F	M	F
Benign	118	55	110	63	130	73	115	70	94	50
Malignant	19	22	10	9	21	12	19	4	20	18
Total	137	77	120	72	151	85	134	74	114	68

C. Effect Levels	Males	Females
No-effect level (NOEL).	100 ppm or 3.64 mg/kg/day	300 ppm or 13.1 mg/kg/day
Low effect level (LEL). Basis Males: mild renal toxicity noted in the 1,000 ppm group; 300 ppm group had decreases in urniary specific gravity and/or protein. Females: mild renal toxicity present in the 1,000 ppm group.	300 ppm or 11.01 mg/kg/day	1,000 ppm or 45.7 mg/kg/day

D. The increased incidence in males and frequency of three specific tumor types (pituitary, thyroid, and brain carcinomas) as reported in the Toxicology Branch 1982 review for EPA accession numbers 244476-244481 were not seen in the present study (EPA accession numbers 255729-255732).

9. Data

A. 3,000 ppm of 2,4-DP acid: Tumors (neoplastic lesions) were not significantly increased when compared to the controls. Both sexes had significant decreases in food consumption throughout most of the study through week 70 (males) and 84 (females). Both sexes had distinct reductions in body weights and increases of water consumption throughout the study. Females showed a decrease in food efficiency. Both sexes had mild anemia, and increases in platelet count were observed in females at weeks 78 and 104.

Other changes determined by blood analyses were: 1) Elevation of the albumin/globin ratio resulting from an increase in albumin and a decrease in globulin for both sexes. 2) Elevation of alkaline phosphatase (males only). 3) A rise in sodium (males at 52 and 78 weeks; females at 26 and 78 weeks). 4) Increased glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and total bilirubin (males at 78 and 104 weeks). Other physiological notations included the following: 1) Decrease in urinary specific gravity and protein at each of the assigned examinations. 2) High incidences of a dark color of the kidney at gross necropsy in both sexes; dark color of the liver in males; and hair loss in females 22/80 compared to 9/80 for controls). 3) Distinct increases in the male kidney weights at the 104th week ($p=.001$). 4) Gross pathology indicated increased ($p=<.05$) mammary gland hypertrophy for females (4/80). 5) Histopathological examination demonstrated: a) Increased incidences of diffuse hepatocellular swelling. b) Increased brown pigment (lipofuscin) deposition in the proximal tubular epithelium of the kidney of both sexes. c) Increased incidences of brown pigment (lipofuscin) deposition in hepatocytes and mineralization in the kidney of males. Males exhibited statistically significant increases in the histological incidence of prostatitis. Females exhibited statistically significant retinal atrophy/degeneration (13/75 compared to 1/75 for controls).

B. 1,000 ppm of 2,4-DP acid: At weeks 78 and 104, females exhibited increased water consumption, and both sexes had decreases in urinary specific gravity and/or protein. At the 104th week, females showed an increase in serum potassium, and males had increased kidney weight. At necropsy, the incidence of a dark color of the kidney significantly increased in either sex (8/80 males and 20/80 females). Histological examination showed significant increases in the incidence of brown pigment deposition in the proximal tubular epithelium of kidneys for both sexes. Males exhibited statistically significant increases in the histological incidence of prostatitis. Gross pathology indicated: 1) Increased ($p=<.05$) mammary gland hypertrophy for females (5/80). 2) Increased plantar region callosity/mass ($p=<.05$). These gross pathology findings were not toxicologically significant.

C. 300 ppm of 2,4-DP acid: At weeks 78 and 104, decreases were seen for males in urinary specific gravity and/or protein. Distinct changes attributable to the diet were not seen for females. Gross pathology indica-

ted (not toxicologically significant): 1) Increased nodule/ mass in the skin of males (p=<.05). 2) Increased subcutis nodule/mass (p=<.05). Histopathology indicated that these lesions were nonspecific, and not dose related.

D. 100 ppm of 2,4 DP acid: Treatment related changes in either sex were not evident.

E. Further Discussions

Body Weights

Statistically significant reductions (p=<.05) in the mean body weights of both sexes attributable to the diets were seen in the 3,000 ppm group (approximately 10%). A reduction of male body weights (1,000 ppm) was seen at weeks 3, 5-15, and 84-94 (2-4%). Females (1,000 ppm) had body weight reductions (p=.05) at weeks 2 and 3. Reductions in body weights (p=≤.05; 2-5%) were seen in males (300 ppm) at weeks 3-20, 26-42, 46-48, 52, and 88-96. Females (300 ppm) had reductions in body weights (p=.05) only at the third week.

Food Consumptions

Compared to the controls, significant decreases (p=≤.05) in food consumption for the 3,000 ppm group were seen at weeks 1-5, 7-9, 11, 16, 21-23, 30, 32, 34, 38, 40, 42, 66, and 84 for females, and at weeks 1-3, 5, 6, 8, 18, 19, 23, 26, 28, 30, 34, 38, 40, 50, 52, 60, 62, 64, 68, and 70 for males. At 1,000 ppm, food consumption increases (p=≤.05) were seen in males for weeks 1, 5, 8, 22, 24, 30, 50, 60, 68, 70, and at weeks 7, 8-14, 16-19, 21, 56, 58, 92-98 for females. For the 300 and 100 ppm groups, sporadic increases/reductions (p=≤.05) were seen throughout most of the study.

Food Efficiencies (body weight gain/food consumption x 100)

Males (3,000 ppm) and both sexes in the other treated groups had no consistent changes in food efficiency. Females had a decrease in food efficiency in the 3,000 ppm group with a 16% lower mean value compared to controls.

Water Consumptions

The increased water consumption in males and females of the 3,000 ppm group and in the females (1,000 ppm group) appear to be treatment related.

Cumulative Mortality

Significant differences were not seen between the treated groups as compared to the controls.

F. Other

Urinalysis

The decreases in the specific gravity and/or protein in males ($\geq$ 300 ppm), and in females ($\geq$ 1,000 ppm) are assumed to be related to nephropathology.

Hematology

Predominantly, decreases were seen at the high dose level in both sexes for hemoglobin, hematocrit, and erythrocytes, and indicated mild anemia. Other hematological data significances appeared sporadically and are not considered to be toxicologically significant.

Other biochemistry

Biochemical changes related to the duration of the treatment were increases in alkaline phosphatase, GPT, and GOT in males in the 3,000 ppm group, and attributable to histological evidence of diffuse hepatocellular swelling. Factors related to treatment were: a) Increased total bilirubin in males (3,000 ppm). b) Increased sodium in both sexes (3,000 ppm). c) Increased potassium in females (1,000 and 3,000 ppm). These values were statistically significant compared to the controls, but may/may not be toxicologically significant. Other biochemical statistically significant changes seen in the treated groups were judged not dose related or inconsistant over time.

Pathology (appendix 2)

Gross

The dark coloring of the kidney (males and females treated with 1,000 ppm or more) and liver (males, 3,000 ppm) are considered to be associated with the reported histology of these tissues. Organ/body weight ratio, and/or organ/ brain weight ratio of the kidney sometimes increased in males in the treated groups and in females treated with 300 ppm or more. An increase of absolute and relative kidney weights was seen only in males (1,000 ppm or more at the 104th week), and it is considered to be related to the brown pigmentation in the proximal tubular epithelium. Other statistically significant changes in other organs were seen in the 3,000 ppm group, but are not reported to be histologically significant.

Histopatholgy

The increased incidences of hepatocellular swelling and brown pigment (lipofuscin) deposition of hepatocytes appear to be effects of the treatment. Statistically significant increases in the incidence of increased brown pigment (lipofuscin) deposition of the proximal tubular epithelium were seen in both sexes of the 1,000 ppm or more groups, and suggests a mild degeneration of the tubular epithelium.

Significant increases in the cumulative incidence of retinal atrophy (13/75: 17.3%) were seen histologically in females (3,000 ppm). The incidence of this lesion in control Fisher rats from other chronic toxicity studies conducted at the Institute Of Environmental Toxicology in Japan was reported to be 8.8-17.5%. Unilateral lesions appeared in 9 of 13 rats (1.3%) of the 3,000 ppm group, and no abnormalities were detected in the opposite retina. This finding appears to be toxicologically insignificant.

Significant increases were seen in the incidence of prostatitis in the 1,000 and 3,000 ppm groups. It was reported that this lesion also appears spontaneously, and there was no distinct morphological difference between control and test groups (1,000 and 3,000 ppm). It appears that this finding is toxicologically insignificant.

G. The study is acceptable as Core: Guideline.

H. The dioxin content and other manufacturing impurites of 2,4-DP formulation should be assessed. Toxicology Branch defers to RCB as to the dioxin content and other impurities of the formulation.

10. Previous Studies Submitted By Union Carbide

18 Month Oncology Study In Swiss-Webster (CD-1) Mice (0, 25, 100, and 300 mg/kg); EPA accession no. 242035-242038.

Toxicology Branch (James Holder) reviewed studies in 1982 submitted by Union Carbide. Included in review was an eighteen month oncology study, which was conducted in Swiss-Webster (CD-1) mice at 0, 25, 100, and 300 mg/kg of 2,4 DP acid. The reviewer concluded that: "Stress toxicity was manifest at 25 and 100 mg/kg doses with liver weights, areas of degeneration and areas of regeneration were observed in the liver increasing hematopoiesis, myelopoiesis, and granulopoiesis which is a typical stress response in aging mice. These increased synetheses (sic)

were accompanied by some anisonormocytosis. At the high dose (300 mg/kg) bile retention, increased. Thus, for general toxicity in this 18-month mouse study: NOEL = 100 mg/kg and LEL = 300 mg/kg. No tumor kinds (benign or malignant) or types (cell-or tissue-specific) were dose related to the feeding of 2,4 DP acid. Noteable is the increased heptomas at the high dose group (18% vs. 7.8% in controls). This response is viewed as weak tumor promotion due to obvious trauma to the liver by 2,4 DP acid at 300 mg/kg. It is concluded that this singular increase in tumors is not a dose-related response of 2,4 DP acid in mice at 300 mg/kg."

24 Month Sprague Dawley Rat Oncology Study (0, 25, 50, 200/ 150 mg/kg or 0, 500, 1,000, 4,000/3,000 ppm); EPA accession no. 244476 - 244481.

Also included in the Toxicology Branch 1982 review by James Holder was a two-year Sprague Dawley rat oncology study at 0, 25, 50, and 200/150 mg/kg (switched at 60 weeks) of 2,4 DP acid in feed. The reviewer stated that the high dose produced pronounced toxicity in liver, kidney, and lymph nodes in both sexes. Males were affected by lung congestion, chronic prostatitis, testicular atrophy, edema, and hyperplasia. These toxic effects were also observed to a lesser extent at the mid-dose level of 50 mg/kg, and were absent at 25 mg/kg. It was concluded from this study that 2,4 DP is a carcinogen in male Sprague Dawley rats because of the following: 1) Increased incidence and frequency in males by dosage of malignant tumor types compared to controls. 2) Increased incidence in males and frequency of three specific tumor types: pituitary, thyroid, and brain carcinomas. 3) A decrease in life span in male rats with pituitary and brain tumors. 4) A shift with dose in the malignant tumor pattern in male controls to the malignant tumor pattern in the male treated groups. The treated groups had 85-86% of pituitary and thyroid malignant tumors whereas the controls had 37% of these tumor types. 5) Increased tumor load with dose (number of tumors/rat) in male rats . 6) Occurrence in both sexes of a rare tumor type such as brain tumors.

11. Core classification: Guideline.

APPENDIX (1)

APPENDIX

5. TEST COMPOUND

Code name:	2,4-DP acid
Chemical name:	2-(2,4-dichlorophenoxy)propanoic acid
Purity:	95% (Lot No. 9/22/80: June 19, 1981 - January 31, 1982)
	95% (Lot No. 10/31/80: February 1, 1982 - June 25, 1983)
Appearance:	white crystalline solid
Melting point:	116 - 117.5°C
Solubility:	slightly soluble in water (0.71 g/l water) soluble in acetone and benzene
Stability:	stable at room temperature
Storage conditions:	kept in dark and cold environment (in a cold room at 4°C)

6. MATERIALS AND METHODS

1) Experimental animals

(1) Experimental animals and reason for selection

Specific pathogen free (SPF) Fischer (F-344) rats of both sexes were purchased from Charles River Japan, Inc. (Shimo-furusawa, Atsugi-shi, Kanagawa).

The rat is a suitable species for chronic toxicity study and was also assigned by the sponsor.

(2) Date of receipt

Male: June 18, 1981

Female: June 11, 1981

(3) Age and body weight at receipt

4 weeks o. age

Male, 60 - 75 g; Female, 50 - 65 g

(4) Acclimatization

Animals were observed daily by veterinarians for a week after receipt. No abnormalities in the general condition were seen in these animals during the acclimatizing period.

(5) Age at initiation of treatment

5 weeks of age.

2) Management of animals

(1) Handling procedures

(a) Environmental conditions

The animals were housed in a barrier-sustained (BS) animal room. When entering the room, workers put on working clothes, caps, and masks for BS-use only, after shower bathing of whole body. All instruments were carried into the room after sterilized by autoclave, etc. The environmental conditions were as follows:

Temperature; 24 ± 1°C

Humidity; 55 ± 5%

Air changes; 12 times per hour

Illumination; 14 hours per day (light on at 5:00 a.m. and off at 7:00 p.m.).

The environmental control data during the study are shown in Appendices 25 and 26.

(b) Racks and cages

Wire-mesh stainless steel cages (width 310 mm x depth 440 mm x height 230 mm) were fixed into movable stainless

steel racks The animals were housed in groups of 5/sex/cage at initiation of treatment. Numbered color tapes indicating the group, sex, and dose level were stuck on racks and each cage. Racks and cages were exchanged once per 2-4 weeks for washed and sterilized ones.

(c) Grouping of animals

Animals were weighed at initiation of treatment. They were allotted by random selection to each dose group as making the body weight distribution approximately equal.

(d) Identification of animals

Animals in a cage were uniquely identified by the marking of a part of the body with saturated picric acid solution in 70% alcohol as follows:

No. 1: Head No. 2: Back No. 3: Hip

No. 4: Head and back No. 5: No mark

(2) Diet

Diet M (Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo) which was obtained from pulverization of pellet diet MF was used for the basal diet. Test diets were supplied ad libitum from stainless steel feeding jars (Towa Kagaku Co., Ltd., Nishikanda, Chiyoda-ku, Tokyo).

Analysis of the basal diet for contaminants listed on Appendix 27 was performed once per 2 to 3 months during the treatment by Oriental Yeast Co., Ltd.

(3) Water

Local tap water (Kodaira-shi) was supplied ad libitum from polycarbonate bottles (Tokiwa Kagakukikai Co., Ltd.,

Ueno, Taito-..u, Tokyo).

Analysis of the water for contaminants listed on Appendix 28 was performed twice a year during the treatment by this facility and The Institute of Food Sciences, Japan Medical Foods Association (Maesawa, Higashikurume-shi, Tokyo).

3) Route of administration and treatment period

2,4-DP acid was incorporated into basal diet and administered to the animals ad libitum for a period of 24 months (104 weeks).

4) Preparation of test diets

Preparation of test diets was performed twice a week. For each dose level, 2,4-DP acid was mixed with a part of basal diet in a mortar. Thereafter, the mixture was put into the rest of basal diet and stirred to obtain the test diet of prescribed concentration by a mixer (Ikeda Rika Co., Ltd., Iwamoto-cho, Chiyoda-ku, Tokyo).

Stability of 2,4-DP acid in the diet was determined by this facility prior to initiation of treatment. It was confirmed that 2,4-DP acid was stable in the diet at least for 7 days (Appendix 29-2). Sample of the test diet of each dose level was analyzed chemically in this facility to ascertain the concentration of 2,4-DP acid in the diets prior to initiation of treatment and monthly during the treatment period. The monthly chemical analysis was performed on samples of 50 grams of the test diets at the last preparation time of each month. The analytical data are shown in Appendix 29.

5) Dose levels and group size

A preliminary 4-week range finding test was conducted at dietary levels of 0, 100, 300, 1,000, 3,000 or 5,000 ppm of 2,4-DP acid to determine the dose levels for the present study. In the preliminary test, a significant inhibition of body weight gain was noted in the groups treated with 3,000 ppm or more and the liver weight significantly increased in male groups treated with 1,000 ppm or more and in female groups treated with 3,000 ppm or more. Based on the results of the preliminary range finding test, the following dose levels were determined:

Dose Level (ppm)	Group Size (Animal Number)	
	Male	Female
0	80 (1 - 80)	80 (1 - 80)
100	80 (81 - 160)	80 (81 - 160)
300	80 (161 - 240)	80 (161 - 240)
1,000	80 (241 - 320)	80 (241 - 320)
3,000	80 (321 - 400)	80 (321 - 400)

Eight animals of each sex from each dose group were killed by design at 26, 52 and 78 weeks of treatment to be subjected to urinalysis, hematology, blood biochemistry, and pathology.

6) Observations

(1) Clinical sign

All animals were observed daily for their clinical signs. When animals showing clinical signs were found, the onset, nature and severity of the symptom, and the duration were

recorded.

(2) Mortality

Animals showing severe toxic signs or marked debility were either removed to separate cages and replaced when recovered or sacrificed to preserve their tissues.

Dead animals were autopsied immediately after discovery in order to minimize the autolysis.

Mortality was expressed as ratios of cumulative number of animals found dead or killed in extremis to the effective number of animals per group. Animals for interim kill were eliminated from the effective number of animals.

(3) Body weight

Body weight of each animal was recorded weekly during the first 26 weeks and biweekly thereafter. Group mean body weights were calculated at each time of measurement.

(4) Food consumption and chemical intake

Food consumption of rats was measured twice a week for 8 cages per group during the treatment. The cages were designated for the measurement at initiation of treatment. Mean daily food consumption per animal was calculated weekly during the first 26 weeks and biweekly thereafter by dividing total food consumption by number of animals in the cage and days for measurement. Group mean chemical intake was calculated from the food consumption and dose level.

(5) Food efficiency

Group mean food efficiency was calculated from the ratio of mean body weight gain to mean food consumption and

recorded.

(2) Mortality

Animals showing severe toxic signs or marked debility were either removed to separate cages and replaced when recovered or sacrificed to preserve their tissues.

Dead animals were autopsied immediately after discovery in order to minimize the autolysis.

Mortality was expressed as ratios of cumulative number of animals found dead or killed in extremis to the effective number of animals per group. Animals for interim kill were eliminated from the effective number of animals.

(3) Body weight

Body weight of each animal was recorded weekly during the first 26 weeks and biweekly thereafter. Group mean body weights were calculated at each time of measurement.

(4) Food consumption and chemical intake

Food consumption of rats was measured twice a week for 8 cages per group during the treatment. The cages were designated for the measurement at initiation of treatment. Mean daily food consumption per animal was calculated weekly during the first 26 weeks and biweekly thereafter by dividing total food consumption by number of animals in the cage and days for measurement. Group mean chemical intake was calculated from the food consumption and dose level.

(5) Food efficiency

Group mean food efficiency was calculated from the ratio of mean body weight gain to mean food consumption and

expressed as percentage.

(6) Water consumption

Water consumption of rats was measured twice a week for 8 cages per group during the entire study. The cages were designated for the measurement at initiation of treatment. Water consumption was calculated weekly during the first 26 weeks and biweekly thereafter by dividing total water consumption by number of animals in the cage and days for measurement.

(7) Urinalysis

Eight animals of each sex from each group at 26, 52 and 78 weeks of treatment were subjected to urinalysis. Urinalysis was performed on all surviving animals at termination of treatment. Urine was sampled before blood sampling by pressing the lumbodorsal region. Specific gravity was determined with Handy Refractometer (Atago Optical Instrument Inc., Hon-cho, Itabashi-ku, Tokyo). Uro-labstix® (Miles-Sankyo Co., Ltd., Ginza, Chuo-ku, Tokyo) was used to examine the urine samples for pH, protein, glucose, ketones, occult blood, and urobilinogen.

(8) Hematology

Eight animals of each sex from each group at 26, 52 and 78 weeks of treatment were subjected to the hematological examinations. The examination was performed on 10 animals of each sex from each group at termination. Animals were anesthetized with ether and laparotomized. Blood was withdrawn through the posterior vena cava into syringes. A part

of the blood sample was poured into a cup with EDTA and used for the hematological examinations.

Differential leukocyte count was calculated by multiplyirg leukocyte count by percentage of each leukocyte type determined on blood smear stained with May-Grünwalds and Giemsa. Reticulocyte count was determined on blood smear stained by supravital staining.

The following parameters except those described above were examined with a hematological autoanalyzer Coulter Counter® Model SP (Japan Scientific Instrument Co., Ltd., Ichiban-cho, Chiyoda-ku, Tokyo):

Parameter	Abbreviation	Unit
Hematocrit	Ht	%
Hemoglobin	Hb	g/dl
Erythrocyte count	RBC	mill.*[1]/cmm
Mean corpuscular volume	MCV	fl*[2]
Mean corpuscular hemoglobin	MCH	pg*[3]
Mean corpuscular hemoglobin concentration	MCHC	g/dl
Platelet count	Platelet	1,000/cmm
Leukocyte count	WBC	1,000/cmm
Differential leukocyte count	Differential count	1,000/cmm
Reticulocyte count	Retics	/1,000 RBC

*1, million 1 mill. = 1(

*2; femtoliter 1 fl = 10^{-15} l

*3; picogram 1 pg = 10^{-12} g

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(9) Blood biochemistry

Eight animals of each sex from each group at 26, 52 and 78 weeks of treatment were subjected to the blood biochemistry. The examination was performed on 10 animals of each sex from each group at termination. With a blood biochemical autoanalyzer Hitachi 726 and Hitachi Flame Photometer 205D (both from Nissei Sangyo Co., Ltd., Nishishinbashi, Minato-ku, Tokyo), the following parameters were determined on serum samples of blood obtained for the hematological examinations:

Parameter (Abbreviation)	Unit	Method
Total protein (TP)	g/dl	Biuret method
Albumin (Alb)	g/dl	BCG method
Globulin (Glob)	g/dl	Calculated; (TP - Alb)
Albumin/globulin ratio (A/G)	___	Calculated; (Alb/Glob)
Alkaline phosphatase (AlP)	U/l	Modified Bessey Lowry method
Lactate dehydrogenase (LDH)	U/l	UV method
Blood urea nitrogen (BUN)	mg/dl	Urease-Indophenol method
Glucose	mg/dl	GOD-POD method

Parameter (Abbreviation)	Unit	Method
Total cholesterol (T.Chol)	mg/dl	Enzyme method
Glutamic oxaloacetic transaminase (GOT)	U/l	UV method
Glutamic pyruvic transaminase (GPT)	U/l	UV method
γ-Glutamyl transpeptidase (GGTP)	U/l	Modified Orlowski method
Total bilirubin (T.Bil)	mg/dl	Azobilirubin method
Direct bilirubin (D.Bil)	mg/dl	Azobilirubin method
Calcium (Ca)	mg/dl	OCPC method
Sodium (Na)	mEq/l	Flame emission photometry
Potassium (K)	mEq/l	Flame emission photometry

(10) Autopsy

Animals subjected to interim kill or moribund kill and all surviving animals at the end of the study were killed by cutting the posterior vena cava and removing the diaphragm under ether anesthesia. Complete autopsy was performed on these animals. Dead animals were autopsied immediately after discovery. Autopsy on the animals subjected to interim kill was performed after 26, 52 and 78 weeks of the treatment. Autopsy on the animals subjected to terminal kill was performed on days 728, 729 and 730 in males and on days 728, 729 and 731 in females.

All the animals were observed carefully on the external

surfaces and 11 orifices; cranial cavi external or cut surfaces of the brain and spinal cord; neck with its associated organs and tissues; thoracic, abdominal, and pelvic cavities and their associated viscera; and the muscular skeletal carcass.

(11) Organ weight

After autopsy, weights of the following organs were recorded for each animal subjected to interim and terminal kills and organ/body weight ratios were calculated:

Brain	Pituitary
Thyroids (with parathyroids)	Heart
Thymus*	Liver
Kidneys	Spleen
Adrenal glands	Testes/Ovaries
Skeletal muscle (M. triceps surae)	

*; Thymus was weighed only at 26 weeks of treatment because physiological regression with age was marked thereafter.

In addition, organ/brain weight ratios were also calculated on the liver, kidney, heart and spleen.

(12) Histopathology

The following organs and tissues from all animals subjected to interim and terminal kills and all of those killed in extremis were preserved in neutral buffered 10% formalin:

All gross lesions (with a border of normal tissue; in case of tumor, with neighboring lymph nodes as possible)
Brain (3 sections)
Spinal cord (cervical, thoracic and lumbar regions)
Eyes and contiguous glands (bilateral)
Pituitary Salivary gland
Thyroids with parathyroids (bilateral)
Heart (2 sections)
Thymus (region of thymus at 52 weeks and later)

Lung (coronary 2 sections with the main stem bronchi and all lobes)
Larynx
Trachea
Esophagus
Stomach (forestomach, glandular region, and pylorus)
Duodenum
Jejunum
Ileum
Cecum
Colon
Pancreas
Liver (2 sections)
Adrenal glands (bilateral)
Kidneys (bilateral)
Urinary bladder
Testes (bilateral)
Epididymides (bilateral)
Prostate
Seminal vesicles
Ovaries (bilateral)
Corpus and cervix uteri
Spleen (2 sections)
Lymph nodes (cervical, mesenteric)
Sciatic nerve
Skin (lumbodorsal region)
Mammary glands (abdominal region)
Thoracic aorta
Head (3 coronary sections including nasal cavity, paranasal sinuses, tongue, oral cavity, nasopharynx, and inner ears)
Bone including marrow (from the sternum, vertebra, femur, and tibio-femoral joint)
Skeletal muscle (M. triceps surae)

On animals found dead during the treatment, the organs described above were preserved when available.

Microscopic examination was performed on preparations stained mainly with hematoxylin and eosin. In addition, other stainings including Prussian blue reaction for iron and Schmorl stain for lipofuscin were also applied to the liver and kidney.

7) Statistical evaluation

Analysis of variance was performed by Student's t test to determine the significance of the results. Fisher's exact probability test was applied to the incidence of pathological data. Mann-Whitney U test was applied to the specific gravity and protein in urinalysis.

APPENDIX (2)

Table 27 - 1 Incidence of major pathological lesions in each designated period in male rats

Findings	Dose group (ppm)	Treatment period (weeks) 0 - 26 fd+ke	26 ik	27 - 52 fd+ke	52 ik	53 - 78 fd+ke	78 ik	79 - 104 fd+ke	104 tk	Total
Gross findings										
Liver: Dark in color	0	0/0[a,b]	0/8	0/0	0/8	0/4	0/8	0/15	0/34	0/80
	100	0/0	0/8	0/2	0/8	0/5	0/8	0/9	0/40	0/80
	300	0/0	0/8	0/0	0/8	0/3	0/8	0/12	0/41	0/80
	1,000	0/0	0/8	0/0	0/8	1/4	0/8	0/11	0/41	1/80
	3,000	0/1	0/8	0/1	5/8*	0/2	1/8	0/13	0/39	6/80*
Kidney: Dark in color	0	0/0	0/8	0/0	0/8	0/4	0/8	1/15	0/37	1/80
	100	0/0	0/8	0/2	0/8	0/5	0/8	2/9	0/40	2/80
	300	0/0	0/8	0/0	0/8	0/3	0/8	2/12	0/41	2/80
	1,000	0/0	0/8	0/0	2/8	1/4	1/8	1/11	3/41	8/80*
	3,000	0/1	0/8	0/1	8/8***	0/2	6/8**	4/13	13/39***	31/80***
Histological findings										
Liver: Diffuse hepato-cellular swelling	0	0/0	0/8	0/0	0/8	0/4	0/8	0/15	0/37	0/80
	100	0/0	0/8	0/2	0/8	0/5	0/8	0/9	0/40	0/80
	300	0/0	0/8	0/0	0/8	0/3	0/8	0/12	0/41	0/80
	1,000	0/0	0/8	0/0	0/8	0/4	0/8	0/11	0/41	0/80
	3,000	1/1	8/8***	1/1	8/8***	1/2	7/8***	8/13***	28/39***	62/80***

[a], No. of rats with lesions; [b], No. of rats examined; ik, interim kill; tk, terminal kill; fd, found dead; ke, killed in extremis;
*p< 0.05, **p< 0.01, ***p< 0.001 (Fisher's exact test), as compared to the controls

Table 27 - 2 Incidence of major pathological lesions in each designated period in male rats

Findings	Dose group (ppm)	Treatment period (weeks) 0 - 26 fd+ke	26 ik	27 - 52 fd+ke	52 ik	53 - 78 fd+ke	78 ik	79 - 104 fd+ke	104 tk	Total
Histological findings										
Liver: Increased brown pigment deposition in hepatic cells	0	0/0[a,b]	0/8	0/0	0/8	0/4	0/8	0/15	0/37	0/80
	100	0/0	0/8	0/2	0/8	0/5	0/8	0/9	0/40	0/80
	300	0/0	0/8	0/0	0/8	0/3	0/8	0/12	0/41	0/80
	1,000	0/0	0/8	0/0	0/8	0/4	0/8	0/11	0/41	0/80
	3,000	0/1	0/8	1/1	6/8**	1/2	4/8*	7/13**	7/39**	26/80***
Kidney: Mineralization	0	0/0	0/8	0/0	0/8	0/4	0/8	0/15	0/37	0/80
	100	0/0	0/8	0/2	0/8	1/5	0/8	0/9	0/40	1/80
	300	0/0	0/8	0/0	0/8	0/3	0/8	0/12	0/41	0/80
	1,000	0/0	0/8	0/0	0/8	1/4	0/8	0/11	0/41	1/80
	3,000	0/1	0/8	1/1	0/8	0/2	1/8	1/13	5/39*	8/80**
Increased brown pigment deposition in proximal tubular cells	0	0/0	0/8	0/0	0/8	0/4	0/8	2/15	1/37	3/80
	100	0/0	0/8	0/2	0/8	0/5	0/8	2/9	0/40	2/80
	300	0/0	0/8	0/0	0/8	0/3	0/8	1/12	1/41	2/80
	1,000	0/0	0/8	0/0	5/8*	1/4	7/8***	6/11*	34/41***	53/80***
	3,000	0/1	0/8	1/1	8/8***	2/2	8/8***	13/13***	39/39***	71/80***
Prostate: Prostatitis	0	0/0	0/8	0/0	0/8	1/4	0/8	2/15	6/37	9/80
	100	0/0	0/8	0/2	0/8	0/5	0/8	0/9	8/40	8/80
	300	0/0	0/8	0/0	0/8	1/3	0/8	0/11	9/41	10/79
	1,000	0/0	0/8	0/0	0/8	1/4	0/8	2/11	15/41*	18/80*
	3,000	0/1	0/8	0/1	0/8	1/2	0/8	1/13	16/39*	18/80*

[a], No. of rats with lesions; [b], No. of rats examined; ik, interim kill; tk, terminal kill; fd, found dead; ke, killed in extremis;
*p< 0.05, **p< 0.01, ***p< 0.001 (Fisher's exact test), as compared to the controls

Table 28 - 1 Incidence of major pathological lesions in each designated period in female rats

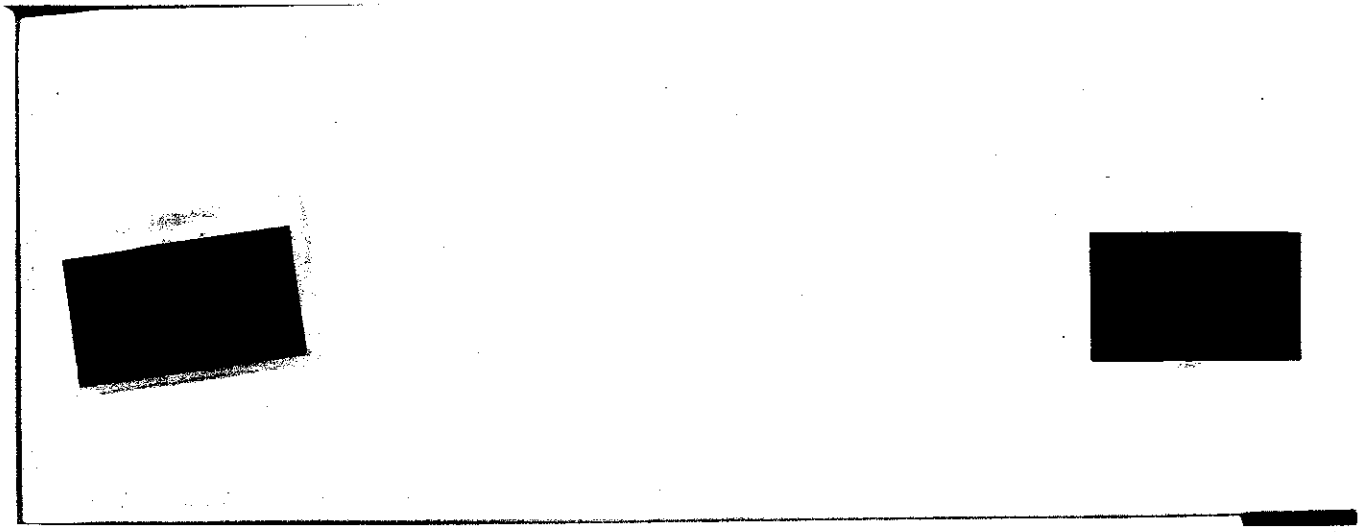
Findings	Dose group (ppm)	Treatment period (weeks) 0 - 26 fd+ke	26 ik	27 - 52 fd+ke	52 ik	53 - 78 fd+ke	78 ik	79 - 104 fd+ke	104 tk	Total
Gross findings										
Kidney: Dark in color	0	0/0[a,b]	0/8	0/1	0/8	0/5	0/8	1/13	1/37	2/80
	100	0/0	0/8	0/0	0/8	1/5	0/8	0/9	1/42	2/80
	300	0/0	0/8	0/0	0/8	0/3	0/8	0/16	5/37	5/80
	1,000	0/0	0/8	0/5	2/8	0/2	0/8	1/8	17/41***	20/80***
	3,000	0/1	0/8	0/1	6/8**	0/7	0/8	6/17	21/30***	33/80***
Skin: Hair loss	0	0/0	0/8	0/1	0/8	0/5	2/8	3/13	4/37	9/80
	100	0/0	0/8	0/0	0/8	0/5	1/8	2/9	6/42	9/80
	300	0/0	0/8	0/0	0/8	0/3	0/8	2/16	6/37	8/80
	1,000	0/0	0/8	0/5	0/8	0/2	1/8	0/8	7/41	8/80
	3,000	0/1	0/8	0/1	0/8	1/7	4/8	8/17	9/30*	22/80**
Histological findings										
Liver: Diffuse hepato-cellular swelling	0	0/0	0/8	0/1	0/8	0/5	0/8	0/13	0/37	0/80
	100	0/0	0/8	0/0	0/8	0/5	0/8	0/9	0/42	0/80
	300	0/0	0/8	0/0	0/8	0/3	0/8	0/16	0/37	0/80
	1,000	0/0	0/8	0/5	0/8	0/2	0/8	0/8	0/41	0/80
	3,000	1/1	8/8***	1/1	8/8***	5/7*	3/8	11/17***	26/30***	63/80***

[a] n, No. of rats with lesions; [b] n, No. of rats examined; ik, interim kill; tk, terminal kill;
fd, found dead; ke, killed in extremis;
*p<0.05, **p<0.01, ***p<0.001 (Fisher's exact test), as compared to the controls

Table 28 - 2 Incidence of major pathological lesions in each designated period in female rats

Findings	Dose group (ppm)	Treatment period (weeks) 0 - 26 fd+ke	26 ik	27 - 52 fd+ke	52 ik	53 - 78 fd+ke	78 ik	79 - 104 fd+ke	104 tk	Total
Histological findings										
Kidney: Increased brown pigment deposition in proximal tubular cells	0	0/0[a,b]	0/8	0/1	0/8	0/5	0/8	7/13	1/37	8/80
	100	0/0	0/8	0/0	0/8	1/5	0/8	0/9*	0/42	1/80*
	300	0/0	0/8	0/0	0/8	0/3	0/8	0/16**	5/37	5/80
	1,000	0/0	0/8	0/5	3/8	0/2	4/8*	3/8	29/41***	39/80***
	3,000	0/1	0/8	1/1	8/8***	5/7*	8/8***	16/17*	30/30***	68/80***
Eye: Retinal atrophy/ degeneration	0	0/0	0/8	0/1	0/8	0/4	0/8	0/9	1/37	1/75
	100	0/0	0/8	0/0	0/8	0/5	0/6	2/8	3/39	5/74
	300	0/0	0/8	0/0	0/7	0/3	0/8	1/12	3/34	4/72
	1,000	0/0	1/8	0/5	0/8	0/2	0/8	0/5	3/41	6/77
	3,000	0/1	0/8	0/1	0/8	0/7	0/5	4/15	9/30**	13/75***

[a] n, No. of rats with lesions; [b] n, No. of rats examined; ik, interim kill; tk, terminal kill; fd, found dead; ke, killed in extremis;
*$p<0.05$, **$p<0.01$, ***$p<0.001$ (Fisher's exact test), as compared to the controls



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Chemical:	**Diclofop (ANSI)**
PC Code:	**110901**
HED File Code	**13000 Tox Reviews**
Memo Date:	**09/11/1985**
File ID:	**TX004654**
Accession Number:	**412-01-0124**

HED Records Reference Center
03/04/2001